Pathogenesis of Swine Influenza Virus (Thai Isolates) in Weaning Pigs

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Introduction
Swine influenza is an acute, highly contagious, respiratory disease that caused by type A influenza virus infection. Subtypes well established in pigs are classic swine influenza virus (SIV) subtype H1N1, H3N2 and H1N2 (2). However pigs can be infected with other subtypes of influenza A viruses which is of substantial importance to the swine industry and to the epidemiology of human influenza (3). In Thailand SIV subtype H1N1 was isolated from pigs with influenza-like symptoms in 1990 (6). However, both (H1N1 and H3N2) SIV subtypes are commonly found among the pig population in the country as revealed by the serological study and virus isolation (1). Recently, a new subtype H1N2 was isolated from six-week-old pigs in August 2005 (1). However the pathogenesis of SIV subtype H3N2 and the new subtype H1N2 in Thailand is not clearly understood. Therefore, the objective of this study is to investigate the pathogenesis of swine influenza virus (Thai isolates) subtype H3N2 and H1N2 in weaning pigs.

Materials and Methods
Fifteen 22-day-old crossbred pigs were obtained and assigned to 3 different groups. Pigs were serologically negative for PRRSV, M. hyopneumoniae and SIV and RT-PCR from the nasal swabs was negative for the M gene of Influenza A virus. Group 1 served as a negative control group contained three pigs using mocked inoculation with the media. The infected groups contained six pigs each and were inoculated with A/Sw/Thailand/CB2/05 (H3N2) intratracheally in group 2 and A/Sw/Thailand/CB1/05 (H1N2) (5 ml of 10^5 TCID_{50}/ml) in group 3 respectively. Two pigs from the infected groups and one pig from the control group were necropsied at 2, 4 and 12 days post-infection (dpi). Pigs were evaluated daily for respiratory disease symptoms and scored as previously described (5). Rectal temperature was measured daily (fever ≥40°C). Nasal swab was done at 0, 1, 2, 3, 4, 5, 7, 10 and 12 dpi. Sera, bronchoalveolar lavage fluid (BALF), tracheal swabs and tissue samples from all organs were collected at each necropsy. At necropsy significant gross lesions of all organs and the percent of lung lesions were recorded. SIV antigen detection was observed in lung tissues by IFA and IHC using anti-influenza A nucleoprotein monoclonal antibody (HB654404 B.V.EUROPEAN VETERINARY LABORATORY, the Netherlands). Virus isolation and identification from sera and BALF were performed (5) and the virus titers (TCID_{50}/ml) were calculated according to Reed and Muench (1938). Sera and nasal swabs were stored at -80°C and tested to evaluate viremia and virus shedding by RT-PCR (8). SIV antibody titers tested by HI assay for subtype H3N2 and H1N2 were also done and sera were absorbed with Trypsin-Heat-Periodate to reduce nonspecific inhibitors before HI testing (11).

Results and Discussion
Fifteen pigs were clinically normal based on hematology and serology which were free from major respiratory pathogens such as PRRSV, M. hyopneumoniae and SIV. In addition, RT-PCR from nasal swabs was negative for influenza A. The results demonstrate that both SIV subtypes (Thai isolates) were able to induce the flu-like symptoms and lesions compatible with viral pneumonia in cranioventral areas and broncho-interstitial pneumonia similar to the previous reports (4, 5, 7, 8). Grossly, the severity of the diseases was greater in the H1N2-infected pigs (Table 1). The H3N2-infected pigs had milder gross lesions and recovered sooner. The severity of the cranioventral pneumonia was observed at 2 dpi and persisted until 12 dpi in the H1N2-infected pigs. H3N2 virus induced mild pneumonia and resolved within 4 dpi. Broncho-interstitial pneumonia consisting of epithelial cells damage, airway plugging, and peribronchial and perivascular infiltration by inflammatory cells were present in both infected groups. The lung is probably the major site of swine influenza virus replication (2) since we did not find any viremic pig at any day of infection. SIV antigen detection was demonstrated by IFA and IHC using specific monoclonal antibodies as early as 2 dpi and found until only 4 dpi (Table 1) in the nuclei of the bronchial and bronchiolar epithelial cells, pulmonary macrophages and pneumocytes in both infected groups. Interestingly, both SIV subtypes can replicate only in the respiratory tract of pigs and shed in nasal secretions. The respiratory symptom and lung pathology in swine influenza-infected pigs depend on the proinflammatory cytokines such as IFN-α, TNF-α, IL-1α and β, and IL-6 in bronchoalveolar lavage.
fluids and the amount of viral load (9). The results showed that both infected group had clinical respiratory signs and higher body temperature than the control group. However, none of the pigs had fever (> 40°C). Cytokines responsible for those are not clearly elucidated yet.

At 2 dpi, the virus titers in BALF of the infected-pigs were between about 10^2-10^3 TCID₅₀/ml and both SIV subtypes started to shed virus in the nasal discharge as early as 2 dpi. However, the H3N2-infected pigs had the PCR-positive results in the nasal swabs only at 2 dpi, but the H1N2-infected pigs had the virus in the nasal swabs until 4 dpi. H1N2 virus was able to cause more severe lesions and be shed from the infected pigs longer. The new subtype, H1N2, is of important for the SIV epidemic and may play a major role in the porcine respiratory disease complex or PRDC in Thailand.

It is generally believed that there is no cross-protection between H1N1 and H3N2 swine influenza viruses. The results demonstrated that H1N2 and H3N2 had no cross HI antibody reaction. Similarly, Van Reeth et al. (2003) clearly demonstrated that anti-influenza virus antibodies in sera from pigs infected with just one virus subtype did not cross-react with another subtype.

### Table 1 Percentages of gross lung lesions

<table>
<thead>
<tr>
<th>dpi</th>
<th>mock*</th>
<th>H3N2**</th>
<th>H1N2**</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Pig 1 (%)</td>
<td>Pig 1 (%)</td>
<td>Pig 2 (%)</td>
</tr>
<tr>
<td>0.0(-)</td>
<td>20.0(+)</td>
<td>2.0(+)</td>
<td>3.0(+)</td>
</tr>
<tr>
<td>4</td>
<td>0.0(-)</td>
<td>2.0(+)</td>
<td>1.0(-)</td>
</tr>
<tr>
<td>12</td>
<td>0.0(-)</td>
<td>0.0(-)</td>
<td>0.0(-)</td>
</tr>
</tbody>
</table>

*One pig from control group was necropsied, **Two pigs from inoculated groups were necropsied, (-) negative antigen detection by IHC, (+) positive antigen detection by IHC

The results of this study may assist in the control and prevention of infection with both SIV subtypes, H3N2 and H1N2. Base on the percentage of cranioventral pneumonia and time of virus shedding, H1N2 virus may play a role in respiratory diseases in weaning pigs in Thailand. More works are needed to be done in the co-infections model with other respiratory organisms and the prevention and control of the SIV-related diseases. In this study, investigations on virus transmissibility in which contact animals are housed together with infected animals were not performed. Therefore, whether these H3N2 and H1N2 subtypes will be transmitted efficiently in the field situation requires further experimental and epidemiologic studies.

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### References